A STUDY OF THE EFFECT OF MANGANESE TOXICITY ON GROWTH AND MINERAL METABOLISM OF YOUNG RATS

by

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A Thesis Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science in Home Economics

Greensboro
May, 1965

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ARTZ, MARTHA DASH. A Study of the Effect of Manganese Toxicity on Growth and Mineral Metabolism of Young Rats. (1965) Directed by: Dr. Aden C. Magee. pp. 71.

The effects of high levels of manganese on growth, hemoglobin levels, liver constituents, and bone mineralization of young male rats were investigated in this study.

Results from this study indicate that high levels of dietary manganese are associated with marked decreases in growth which could be partially alleviated by the addition of 10% of distiller's dried solubles. The data suggest that a high level of protein may accentuate, rather than reduce, the effects of manganese toxicity on growth in rats under certain conditions.

Rats fed excessive dietary manganese showed marked decreases in liver deposition of iron. Since results indicate that this effect can be overcome by the addition of iron to the high manganese diet, the supplemental iron could be alleviating the effect of manganese by supplying the animal with an available form to replace that lost by the action of manganese.

Excessive manganese in the diet resulted in sharp decreases in hemoglobin concentration which could be prevented by the addition of copper and iron to the high manganese diet.

The manganese content of the bones and livers of the animals fed high levels of this mineral was significantly increased. In this study, the addition of calcium and phosphorus was the only supplement capable of preventing this

marked increase in manganese deposition.

Results from this study showed that feeding high levels of manganese had no adverse effect on bone mineralization with respect to calcium, phosphorus, and magnesium deposition.

ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation to Dr. Aden Magee for his guidance, understanding, and patience throughout the direction of this study. Gratitude is also expressed to the members of the advisory committee, Miss Marguerite Felton, Mrs. Mary Dickey, Miss Louise Lowe, for their helpful suggestions, and to Miss Nena Philbrick for her technical assistance.

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CHAPTER I

INTRODUCTION

The essential trace minerals in animal nutrition have been so designated because they have been found to perform vital functions and/or to affect living processes. One of these elements which has been studied is manganese and interest in this particular mineral was stimulated by the revelation that inadequate intakes caused perosis and nutritional chondrodystrophy in poultry. Subsequent studies by various workers further established the necessity of manganese for growth, skeletal development, reproductive performance, and the functioning of the central nervous system.

Although manganese is an essential dietary nutrient for animals, excessive intakes of this mineral have been shown to be antagonistic to other dietary components. Since forage crops and mineral supplements frequently contain high levels of manganese, complete information concerning the biological effects of excessive dietary intakes of this mineral on the animal body is of scientific importance.

Investigators have observed that a number of biological changes occur in animals receiving high or excessive intakes of manganese. Massive consumption of this element

has been shown to interfere with the retention and utilization of phosphorus, calcium, copper, magnesium, and iron.

In man, manganese toxicity has been characterized by extrapyramidal manifestations which are occasionally accompanied by a type of pneumonitis. This disease has been observed among miners following a chronic inhalation of manganese dusts. The condition occurs even though the body appears to maintain the actual tissue concentration of manganese within normal limits. The same process seems to be responsible for the rapid control of this element and for the degenerative disease; however, the mechanisms involved are not understood.

Since the accumulation of evidence demonstrates that an excessive intake of manganese affects the physiological responses within the animal body, further study is necessary for the clarification of the biological activity of this mineral.

CHAPTER II

REVIEW OF LITERATURE

Within recent years, nutritionists have become interested in nutrient imbalances and interrelationships. Of particular interest have been the imbalances and interrelationships that exist between minerals. Recent research findings indicate that high dietary levels of certain mineral nutrients have profound biological effects on various animal species. Manganese is one of those minerals which has attracted the attention of some researchers. This element is omnipresent in all food stuffs, it is found in steady concentration in the tissues of mammals (1), and several research studies (2, 3) have established that physiological functions exist for this nutrient. Manganese is widely used in mineral supplements and occasionally very high levels of this mineral occur in forages which are used for animal consumption.

At present actual dietary requirements for manganese have not been determined, but in 1937 Heller and Penquite (4) reported that a dietary level of .48% of manganese was highly toxic to young chicks. Gallup and Norris (5) found that the growth of hens was not affected by a level of 0.1% of manganese in the diet while dietary levels of

0.1% - 0.2% of manganese were not sufficient for the growth of rats (6). Grummer et al. (7), however, reported that a level of 0.05% of dietary manganese retarded growth of young pigs.

Fore and Morton (1) have shown that bone seems to be the site of highest manganese concentration with the liver as another possible site of storage. Gallup et al. (8) demonstrated that the ingestion of large amounts of dietary manganese was followed by the development of elevated concentrations of this mineral in the liver.

Gubler et al. (9) reported that the addition of a high level of manganese to the diets of rats was associated with no change in the liver copper levels; however, these researchers postulated that manganese formed a complex with copper which resulted in the latter mineral being unavailable for use by the animal body. They also postulated that manganese blocked the action of certain copper-containing enzymes. Simultaneous administration of large amounts of copper and manganese resulted in a marked increase in total body copper level even though a microcytic hypochromic anemia still existed.

Saltman et al. (10) found that manganese completely inhibited the uptake and release of iron by liver slices. This investigation suggested the existence of a common pathway for both these minerals. Further evidence of a manganese-iron antagonism has been shown in lambs (11), in

rabbits, and in pigs (12).

Diets containing supplements up to 5000 p.p.m. of manganese resulted in decreased concentrations of iron in the livers, spleens, and kidneys of lambs with normal hemoglobin levels. Hemoglobin regeneration was severely retarded and serum iron levels were decreased, however, in anemic lambs which were fed diets containing 1000 or 2000 p.p.m. of manganese (11). Depressed hemoglobin formation in rabbits and pigs receiving diets supplemented with 1250 and 2000 p.p.m. of manganese was alleviated with supplements of 400 p.p.m. of iron (12). Matrone et al. (12) have theorized that manganese interferes with iron absorption rather than with hematopoiesis. The mechanism of this interference is unknown.

In addition to copper and iron, several studies have indicated the possibility of interrelationships between manganese and other mineral nutrients. Levels of manganese in excess of 2000 p.p.m. have been shown to interfere with phosphorus retention in rats (6). Chornock et al. (13) reported that a level of 2000 p.p.m. of manganese consumed by rats resulted in marked increases in fecal calcium losses, severe rachetic conditions, and a negative phosphorus balance. Reid et al. (14) demonstrated that a manganese sulfate supplement interfered with calcium metabolism in cattle. The deleterious effects of high levels of magnesium on manganese metabolism in turkey poults could be

overcome by increasing the manganese level of the diet (15). Recently, Magee[1] obtained data which indicated a possible relationship between manganese and zinc.

Although these studies indicate that manganese interferes, in some way, with the growth mechanism of animals and that the effects of excessive manganese in the diet are fairly extensive, the mechanisms involved have not been determined.

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[1]A. C. Magee, 1964. Unpublished data.

CHAPTER III

EXPERIMENTAL PROCEDURES

The objectives of this study were to investigate the nature of the interference of high levels of manganese (a) on growth and hemoglobin levels of young rats, (b) on the metabolism of copper, iron, zinc, and manganese, and (c) on bone mineralization.

Since this study consisted of three experiments, procedures pertaining to a particular experiment will be discussed separately. Several procedures which are common to all of the experiments will be discussed in the following paragraphs.

Young male rats[1] of the Sprague-Dawley strain were used for all of the experiments in the study. The rats were housed in individual wire-bottom cages and were given free access to food and water. Each experiment lasted four weeks. The animals in each experiment were randomized into replications according to initial body weights. Test treatments utilized in a particular experiment were randomly assigned to individual cages within a replication.

The composition of the basal or control diet used

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[1]Sprague-Dawley rats purchased from Holtzman Company, Madison, Wisconsin.

throughout the study is shown in Table 1. Materials tested were added to the basal diet at the expense of starch.

At the termination of each experiment, randomly selected rats from each test diet were sacrificed. Livers and femurs from each animal were removed for subsequent mineral analyses.

A small portion of each liver was weighed separately on an analytical balance and dried in an oven at 35° C. for dry weight data. The remainder of each liver was prepared for mineral analyses by wet ashing with nitric and perchloric acids on a hot plate. The ash of each liver sample was dissolved in 3 ml. of 0.6N HCl and brought to a volume of 100 ml. with redistilled water.

Copper and iron determinations of the liver samples were made by the methods of Parks et al. (16) and Kitzes et al. (17), respectively, as modified by Matrone et al. (18). In general, manganese and zinc contents were determined by means of an atomic absorption spectrophotometer.[2] The exception occurred in Experiment 1 in which zinc was determined by the method of McCall et al. (19).

After the femurs were removed, they were kept in a freezer until they were prepared for mineral analyses. At the time of preparation, the flesh was removed from the bone, and the bones were dried in an oven at 35° C. and weighed

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[2]Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer, The Perkin-Elmer Corporation, Norwalk, Connecticut.

TABLE 1

COMPOSITION OF THE BASAL DIET

Constituents	Per cent
Casein[a]	19
Corn starch[b]	62
Vegetable fat[c]	10
Mineral mix[d]	4
Vitamin mix[e]	2
Cellulose[f]	2
Cod liver oil[g]	1

[a]Vitamin Test Casein, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[b]Globe Easy-flow Corn Starch 3367, Corn Products Sales Company, Greensboro, North Carolina.

[c]Crisco, Procter and Gamble Company, Cincinnati, Ohio.

[d]Salt Mixture W, Nutritional Biochemicals Corporation, Cleveland, Ohio. The composition of this salt mixture is listed as: (in per cent) $CaCO_3$, 21.00; $CuSO_4 \cdot 5H_2O$, 0.039; $FePO_4 \cdot 2H_2O$, 1.470; $MnSO_4$, 0.020; $MgSO_4$, 9.000; $KAl(SO_4)_2 \cdot 12H_2O$, 0.009; KCl, 12.000: KH_2PO_4, 31.000; KI, 0.005; NaCl, 10.500; NaF, 0.057; and $Ca_3(PO_4)_2$, 14,900.

[e]Each 100 gm. of vitamin mix contained (in milligrams): 0.1% vitamin B_{12} (with mannitol), 0.1; biotin, 1; folic acid, 5; thiamine·HCl, 25; pyridoxine·HCl, 25; 2 methyl-naphthoquinone, 50; riboflavin, 50; nicotinic acid, 50; Ca pantothenate, 150; p-aminobenzoic acid, 500; (in grams) inositol 5; Choline chloride, 7.5; DL-methionine, 30; and corn starch, 56.6. All vitamins and methionine were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[f]Alphacel, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[g]Cod liver oil, E. R. Squibb and Sons, New York. This cod liver oil is listed to contain 1700 U. S. P. units of vitamin A and 170 U. S. P. units of vitamin D per 1 gram.

on an analytical balance. The bones were wet ashed by the method previously outlined. The ash of each bone sample was dissolved in 3 ml. of 0.6N HCl and brought to a volume of 25 ml. with redistilled water. Manganese was determined on the original dilution. A second dilution was made for the calcium, phosphorus, and magnesium determinations. Phosphorus was determined by the method of Simonsen et al. (20). In Experiment 2, calcium and magnesium determinations were made by the methods of Weybrew et al. (21) and Simonsen et al. (22), respectively. Calcium and magnesium determinations were made by means of an atomic absorption spectrophotometer in Experiment 3.

A randomized block design was used for each experiment, and all data were subjected to an analysis of variance. Statements of significance are based on odds of at least 19 to 1 ($p \leq 0.05$). Least significant difference (L.S.D.) values were calculated for each experiment to give an indication of the difference between two treatment means which was required to show significance in a particular experiment.

Experiment 1

The purpose of this experiment was to compare the effects of high levels of manganese from various sources on growth, hemoglobin levels, and liver copper, iron, zinc, and manganese deposition. A level of 1.0% of manganese in the forms of carbonate, chloride, and sulfate was

incorporated into separate diets. Only liver samples were taken from randomly selected animals at the end of the experiment.

Experiment 2

The purpose of this experiment was to compare the effects of various levels of manganese on the growth and on hemoglobin levels, the deposition of copper, iron, manganese, and zinc in the liver, and the levels of calcium, phosphorus, magnesium, and manganese in the bones. The levels of dietary manganese supplements, in the sulfate form, utilized in this experiment were 0.25%, 0.5%, and 1.0%.

At the end of the experimental period, liver and bone samples were removed from randomly selected animals on each experimental diet for subsequent mineral analyses.

Experiment 3

One of the techniques commonly used to study the nature of the effect of a mineral toxicity on the animal body has been to attempt to alleviate the adverse conditions caused by the mineral excess by adding various supplements to the diet. There is the possibility that some of the types of supplements used to alleviate the effects of a mineral toxicity, such as zinc, could also alleviate the adverse effects of manganese toxicity. The purpose of this experiment was to study the responses of manganese-fed rats

to 10% supplemental levels of distiller's dried solubles,[3] casein, soybean meal, blood albumin, alpha protein,[4] and lactalbumin, a supplement of 0.8% calcium + 0.8% phosphorus, and a supplement of 0.02% copper + 0.04% iron.

After the four week experimental period, the livers and femurs of randomly selected animals on each diet were removed and prepared for mineral analyses.

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[3]Furnished by the Distillers Feed Research Council and the John F. Young Company, Cincinnati, Ohio.

[4]Nutritional Biochemicals Corporation, Cleveland, Ohio.

CHAPTER IV

RESULTS AND DISCUSSION

Detailed data gathered from this investigation are presented in Appendix A, Tables 1-26.

Experiment 1

The addition of 1.0% of manganese to diets of young rats resulted in highly significant decreases ($p \leq 0.01$) in weight gains, hemoglobin levels, and liver iron levels, and in increases in liver manganese levels which were significant at the 1% level of probability (Table 2). In general, the presence of extra manganese in the diet was associated with increases in liver copper and zinc deposition. These increases in copper and zinc concentrations in the manganese-fed rats, however, were not statistically different from those levels observed in the control animals. Although there were some variations in the responses of the animals to the three manganese compounds, the effects of these three sources of manganese were similar.

Experiment 2

As the level of added manganese increased, there were progressive decreases in weight gains, hemoglobin concentrations, liver iron levels, and liver zinc levels

TABLE 2

RESPONSE OF RATS TO A HIGH LEVEL OF DIETARY MANGANESE FROM THREE SOURCES (EXPERIMENT 1)

Source of Manganese	Level of Added Manganese	Weight Gain at 4 Weeks[a]	Hemoglobin Level[a]	Liver Constituents[b]			
				Cu	Fe	Mn	Zn
	%	gm.	gm./100 ml. blood	mcg./gm. dry weight			
None	--	176	12.07	21.80	357.28	6.16	45.52
$MnSO_4 \cdot H_2O$	1.0	89	8.06	31.56	182.63	74.21	82.26
$MnCl_2 \cdot 4H_2O$	1.0	94	6.44	18.00	120.52	38.01	55.88
$MnCO_3$	1.0	28	6.59	31.48	119.99	29.31	57.92
$L.S.D._{0.05}$[c]		28	1.23	15.47	67.02	23.03	46.26
$L.S.D._{0.01}$		38	1.68	22.23	96.30	33.08	66.46

[a]Each figure represents the mean of eight animals.

[b]Each figure represents the mean of four animals.

[c]Least significant difference at specified probability levels.

(Table 3). Increasing levels of manganese in the diet were also associated with marked increases in liver manganese levels. In this experiment the addition of extra manganese to the diets of young rats had no apparent effect on copper concentrations in the livers.

A level of 1.0% of extra manganese was associated with a highly significant increase ($p \leq 0.01$) in bone calcium deposition (Table 4). Increasing levels of added manganese were also associated with progressive decreases in bone magnesium deposition and marked increases in bone manganese and phosphorus levels.

Experiment 3

Mean growth and hemoglobin levels are shown in Table 5. Liver data appear in Table 6 and bone data, in Table 7. The effect of 1.0% of manganese on weight gains, hemoglobin concentrations, liver iron and manganese levels, and bone manganese levels was similar to the results obtained in Experiments 1 and 2. The addition of 1.0% of manganese had no significant effect on liver copper and zinc levels or on bone calcium, phosphorus, and magnesium levels in this experiment.

The addition of 10% of distiller's dried solubles to the high manganese diet partially alleviated the adverse effect of manganese on weight gain. The addition of all other supplements, with the exception of soybean meal, appeared to accentuate the severity of the effect of

TABLE 3

WEIGHT GAINS, HEMOGLOBIN LEVELS, AND LIVER MINERAL LEVELS OF RATS CONSUMING DIETS CONTAINING VARIOUS LEVELS OF MANGANESE (EXPERIMENT 2)

Level of Added Manganese	Weight Gain at 4 Weeks[a]	Hemoglobin Level[a]	Liver Constituents[b]			
			Cu	Fe	Mn	Zn
%	gm.	gm./100 ml. blood	mcg./gm. dry weight			
0.00	204	12.98	9.50	222.75	3.82	67.07
0.25	190	11.94	9.23	129.75	7.68	63.38
0.50	191	10.07	7.74	88.13	11.47	56.05
1.00	95	7.60	9.27	57.63	21.84	47.97
$L.S.D._{0.05}$[c]	15	.60	1.93	30.15	7.92	13.75
$L.S.D._{0.01}$	20	.81	2.63	41.04	10.79	18.71

[a]Each Figure represents the mean of eight animals.

[b]Each figure represents the mean of four animals.

[c]Least significant differences at specified probability levels.

TABLE 4

EFFECTS OF INCREASING LEVELS OF DIETARY MANGANESE ON MINERAL DEPOSITION IN THE BONES OF YOUNG RATS[a] (EXPERIMENT 2)

Level of Manganese	Bone Constituents			
	Ca	Mg	Mn	P
%	mg./gm. dry weight			
0.00	1046.82	3.51	0.012	88.27
0.25	728.04	3.32	0.013	72.94
0.50	953.54	3.20	0.014	91.28
1.00	2542.70	1.92	0.028	155.45
$L.S.D._{0.05}$[b]	851.84	0.60	0.001	27.96
$L.S.D._{0.01}$	1159.41	0.81	0.002	38.05

[a]Each figure represents the mean of eight animals.

[b]Least significant difference at specified probability levels.

TABLE 5

EFFECTS OF VARIOUS SUPPLEMENTS ON WEIGHT GAINS AND HEMOGLOBIN LEVELS OF RATS FED HIGH LEVELS OF DIETARY MANGANESE[a] (EXPERIMENT 3)

Diet	Weight Gain at 4 Weeks	Hemoglobin Level
	gm.	gm./100 ml. blood
Basal	181	12.50
1.0% Mn	128	8.75
1.0% Mn + Distiller's dried solubles	153	8.66
1.0% Mn + 0.8% Ca + 0.8% P	110	8.73
1.0% Mn + 10% Soybean meal	136	7.74
1.0% Mn + 10% Extra casein	92	8.14
1.0% Mn + 10% Alpha protein	47	8.88
1.0% Mn + 10% Lactalbumin	61	8.43
1.0% Mn + 10% Blood albumin	84	8.77
1.0% Mn + 0.02% Cu + 0.04% Fe	39	12.85
$L.S.D._{0.05}$[b]	34	1.29
$L.S.D._{0.01}$	45	1.73

[a]Each figure represents the mean of six animals.

[b]Least significant difference at specified probability levels.

TABLE 6

EFFECTS OF VARIOUS SUPPLEMENTS ON LIVER MINERAL DEPOSITION OF RATS FED HIGH LEVELS OF DIETARY MANGANESE (EXPERIMENT 3)

Diet	Liver Constituents			
	Cu	Fe	Mn	Zn
	mcg./gm. dry weight			
Basal	17.49	301.21	4.69	73.51
1.0% Mn	19.41	96.80	29.71	81.87
1.0% Mn + 10% Distiller's dried solubles	20.73	84.53	18.29	71.56
1.0% Mn + 0.8% Ca + 0.8% P	16.79	87.78	9.68	82.57
1.0% Mn + 10% Soybean meal	13.48	85.91	21.57	79.60
1.0% Mn + 10% Extra casein	20.55	92.20	19.33	103.19
1.0% Mn + 10% Alpha protein	37.87	99.86	41.42	148.60
1.0% Mn + 10% Lactalbumin	27.77	108.86	31.28	88.68
1.0% Mn + 10% Blood albumin	18.37	96.57	35.98	135.08
1.0% Mn + 0.02% Cu + 0.04% Fe	1005.20	294.12	35.09	150.63
$L.S.D._{0.05}$[b]	70.86	30.60	11.59	92.83
$L.S.D._{0.01}$	95.68	41.32	15.66	125.36

[a]Each figure represents the mean of four animals.

[b]Least significant difference at specified probability levels.

TABLE 7

EFFECTS OF VARIOUS SUPPLEMENTS ON MINERAL DEPOSITION IN THE BONES OF YOUNG RATS FED HIGH LEVELS OF DIETARY MANGANESE (EXPERIMENT 3)

Diet	Bone Constituents[a]			
	Ca	Mg	Mn	P
	mg./gm. dry weight			
Basal	214.40	1.34	0.014	65.51
1.0% Mn	219.37	1.60	0.037	63.21
1.0% Mn + 10% Distiller's dried solubles	209.33	1.56	0.021	48.23
1.0% Mn + 0.8% Ca + 0.8% P	224.27	1.48	0.020	31.30
1.0% Mn + 10% Soybean meal	189.94	1.56	0.024	57.79
1.0% Mn + 10% Extra casein	197.52	1.92	0.026	46.55
1.0% Mn + 10% Alpha protein	146.63	1.67	0.052	80.29
1.0% Mn + 10% Lactalbumin	160.12	1.44	0.043	47.18
1.0% Mn + 10% Blood albumin	175.52	1.79	0.040	42.87
1.0% Mn + 0.02% Cu + 0.04% Fe	176.45	1.72	0.036	56.19
$L.S.D._{0.05}$[b]	44.77	0.45	0.010	28.51
$L.S.D._{0.01}$	61.33	0.62	0.014	39.05

[a]Each figure represents the mean of three animals.

[b]Least significant difference at specified probability levels.

manganese on weight gain.

The addition of 0.02% of copper + 0.04% of iron completely alleviated the adverse effect of manganese on hemoglobin levels and on the deposition of iron in the liver. Animals receiving the high manganese diet supplemented with copper and iron had liver copper levels which were approximately 60 times greater than those found in the control animals. The addition of the alpha protein supplement to the high manganese diet resulted in an additional increase in liver manganese deposition which was significantly higher ($p \leq 0.05$) than the level of manganese deposited in the livers of the animals receiving only manganese. The addition of either casein, alpha protein, blood albumin, or copper plus iron supplements to the high manganese diet resulted in some increase in zinc deposition in the liver. These increases, however, were not significantly different from the liver zinc levels observed in the control animals. In this experiment the presence of 1.0% of added manganese to the diet of young rats had no significant effect on the deposition of calcium, phosphorus, and magnesium in the bones of the animals but was associated with a highly significant increase ($p \leq 0.01$) in manganese deposition in the bones.

Analysis of the bone data gathered from this experiment also revealed that some of the supplements added to the high manganese diet were associated with significant changes

in bone mineral deposition. The presence of alpha protein in the high manganese diet was associated with a level of bone manganese which was significantly higher than that found in the non-supplemented manganese-fed rats. Manganese-fed animals supplemented with alpha protein or lactalbumin had bone calcium levels which were significantly lower ($p \leq 0.01$) than those found in the control animals. The addition of a calcium plus phosphorus supplement to the high manganese diet resulted in a level of bone phosphorus which was significantly lower ($p \leq 0.05$) than the bone phosphorus level of the control animals. Supplements of extra casein and blood albumin were associated with bone magnesium levels which were significantly higher ($p \leq 0.05$) than those of the control animals.

CHAPTER V

GENERAL DISCUSSION

Results of this study indicated that the overall effects of high levels of dietary manganese from various chemical sources on young rats were similar. There were differences in the degree of response of the animals to diets containing the different manganese compounds, but the reasons for these differences were not apparent. There is the possibility that manganese from the three compounds used in this study was not absorbed into the animal body to the same extent. If this supposition is true, differences in animal responses would have been expected. Underwood (23), however, states that there were no differences in responses among poultry maintained on diets supplemented with the oxide, chloride, sulfate, or carbonate forms of manganese.

Although the results of this study confirm earlier studies that high levels of dietary manganese are associated with significant decreases in the growth of young rats, the exact nature of the interference of manganese with the growth mechanism(s) remains a mystery. The addition of a high manganese level to the diet could render the diet so unpalatable that the rats refuse to eat it. If an animal

did not eat, this would suggest that the depressing effect of manganese on growth was caused largely by reduced food consumption. On the other hand, there is the possibility that the depressing effect on growth is the result of an interference with some growth mechanism(s) and that the depression in feed consumption is a result of this interference. Results of this study which show that a supplement of distiller's dried solubles will partially alleviate the subnormal growth associated with high levels of dietary manganese, however, suggest that some necessary growth factor(s) is involved. These findings could be interpreted to mean that manganese, in some way, interferes with or eliminates some factor(s) necessary for growth and that the distiller's dried solubles furnishes this factor. Results of this investigation revealed that the distiller's dried solubles supplement gave better protection against growth depression caused by a high manganese diet than did supplements of other protein sources furnishing equal or greater amounts of protein to the diet. These data suggest that the manganese toxicity alleviating factor is not dietary protein per se. The fact that increasing the level of casein in the diet of the manganese-fed rats from 19% to 29% resulted in a further growth depression suggests that a high protein level may accentuate the effect of manganese toxicity under certain conditions.

Results of this study confirm the findings of Gubler

et al. (9) and Hartman et al. (11) that high levels of dietary manganese intake are associated with marked decreases in liver iron levels. The fact that the adverse effect of manganese on iron deposition in the liver was completely alleviated by iron supplementation indicates a possible iron-manganese interrelationship existing within the animal body. The nature of the interference of manganese with iron metabolism is not apparent from the data collected in this study. Hartman et al. (11) suggested that excessive manganese either converted iron to a form which was physiologically unavailable to the animal or in some manner antagonizes the enzyme systems that oxidize or reduce iron at the site of absorption. Results of Judd (24) indicated that manganese interfered with iron absorption by blocking the mechanism involved in the lowering of the mucosal block. If the main effect of manganese on iron is to convert the iron into a form that is unavailable to the animal, the iron supplement could be alleviating the effect of manganese by supplying the animal with an available form of iron to replace that which has been lost by the action of manganese.

Hartman et al. (11) found that high levels of dietary manganese, in the sulfate form, resulted in marked increases in liver copper levels in lambs, and the results of one experiment of this study are in agreement with these findings. Gubler et al. (9), however, reported that excess

manganese, in the chloride form, had no apparent effect on the deposition of copper in the livers of rats. In this study, excessive manganese, in the chloride form, was found to have no apparent effect on liver copper deposition. The overall results of this study, however, do not give a clear indication of the exact effect of manganese on copper metabolism. The marked increase in liver copper levels in manganese-fed rats supplemented with copper observed in this study is similar to the results of Gubler et al. and indicates the possibility of a copper-manganese interrelationship in the animal body.

Excess manganese in the diet resulted in marked decreases in hemoglobin concentrations in young rats which could be alleviated with a copper and iron supplement. Matrone et al. (12) reported that supplements of iron overcame the depressing effect of manganese on the hemoglobin formation of baby pigs. Gubler et al. (9) found that supplements of copper did not completely alleviate the adverse effect of manganese on hemoglobin formation in rats. Iron supplementation probably furnished iron needed for hemoglobin formation to replace that lost by the adverse effect of manganese since the results of Hartman et al. (11) indicated that manganese interfered with iron absorption rather than with hematopoiesis.

The adverse effect of excessive manganese on bone mineralization reported by Chornock et al. (13) was not

observed in this study. The overall results of this investigation revealed no apparent effect on bone calcium, phosphorus, and magnesium levels.

Excessive manganese in the diets of young rats was associated with marked increases in the deposition of this mineral in the bones and the livers. With the exception of calcium and phosphorus, none of the supplements tested in this study prevented the marked increase in tissue manganese deposition. Excess dietary calcium and phosphorus have been reported to increase the dietary requirements of manganese (23, 25) and there is the possibility that the added calcium and phosphorus facilitated the removal of some of the excessive manganese from the animals fed this ration in this experiment. If this is true, the amount of excessive manganese that would be available for absorption by the animal would be decreased, and one would expect some change in manganese deposition in certain body tissues such as the liver and bone.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

The purpose of this study was to investigate the nature of the interference of high levels of dietary manganese on growth, hemoglobin levels, bone mineralization, and metabolism of copper, iron, zinc, and manganese in young rats.

Specific experiments were designed (1) to determine the changes which occur in young rats receiving diets containing high levels of manganese from various sources, (2) to study the effects of different levels of dietary manganese on the animal body, and (3) to investigate the possibility of alleviating the effects of manganese toxicity by the addition of supplements from various sources.

The criteria used to measure the response of animals to individual treatments were weight gain, hemoglobin levels, bone levels of calcium, magnesium, manganese, and phosphorus, and liver content of copper, iron, manganese, and zinc. All data were analyzed by standard statistical procedures.

Results from the first experiment showed that the addition of high levels of manganese to the diets of young rats produced marked decreases in weight gain, hemoglobin

levels, and liver iron deposition. Manganese and zinc retention were increased in all cases. In general, results from feeding all forms of manganese were similar.

Data from the second experiment indicate that a supplement of 1.0% of manganese resulted in a significant loss of weight. Hemoglobin and iron levels were decreased and liver manganese levels were increased with each elevation in the level of manganese consumed. Although copper retention was reduced when manganese comprised 0.5% of the diet, this element was increased when the other levels were fed. Zinc retention was increased by feeding manganese in the first experiment; however, the opposite effect was produced in this experiment.

Bone levels of calcium were decreased at the 0.25% and 0.5% levels of manganese intake and sharply elevated at the 1.0% level. This level was also associated with a marked decrease in bone magnesium. The concentration of phosphorus in the bone varied with a decrease at the 0.25% level, and a sharp increase at the highest level of manganese intake. Bone deposition of manganese increased as intake rose, indicating that the animal body absorbs this mineral in proportion to intake.

The results from the experiment using various supplements to alleviate the adverse effects of manganese toxicity on growth indicate that additional protein, with the exception of distiller's dried solubles and soybean meal, produces

a further deleterious effect. Although a supplement of copper + iron maintained hemoglobin levels and liver iron deposition, this supplement was associated with a marked decrease in growth. This supplement was also responsible for a high increase in liver copper deposition. Addition of calcium + phosphorus was responsible for preventing the large increases of manganese evident in all other supplemented diets. Extra casein, alpha protein, blood albumin, and copper + iron supplements were associated with a marked increase in liver zinc deposition. With the exception of manganese, bone mineralization was not significantly affected by the supplemented diets.

Recommendations for Additional Investigations

The functions ascribed to manganese are increasing in number with the widespread accumulation of information and experience concerning this mineral. Evidence that excesses of manganese produce adverse effects on other dietary components has stimulated research to determine the exact nature of these interrelationships.

Results from this study have not indicated any clear cut effect of high levels of manganese on the utilization of calcium and phosphorus by the rat. The data, however, show that the calcium plus phosphorus supplement seems to effect a decrease in manganese retention. This finding suggests that an interrelationship may exist among these minerals.

Metabolic studies could be made to further investigate this possibility.

Although a high level of dietary manganese seems to have no effect on liver deposition of copper, liver iron deposition and hemoglobin levels are sharply reduced. Data from this study show that these levels are returned to those of the control animals when a copper plus iron supplement is fed. The interference appears to exist between the high level of manganese in the diet and the utilization of iron. Results from this study suggest the possibility that high levels of manganese may also interfere with enzymes such as cytochrome oxidase, catalase, and succinic dehydrogenase which require iron as a cofactor. If manganese toxicity results in an iron deficiency, then it is possible that iron-containing enzymes would be adversely affected by high levels of dietary manganese. It would be of interest to conduct enzymatic studies to obtain further information.

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APPENDIX A

GROWTH, HEMOGLOBIN, TISSUE MINERAL DATA

TABLE 1

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 1 ON GROWTH OF RATS

Replications	Treatments			
	Basal	1.0% Mn ($MnSO_4 \cdot H_2O$)	1.0% Mn ($MnCl_2 \cdot 4H_2O$)	1.0% Mn ($MnCO_3$)
	4 weeks weight gain (gm.)			
1	189	85	189	30
2	213	101	73	50
3	185	84	76	2
4	107	83	85	5
5	175	102	81	4
6	202	77	94	40
7	146	83	76	59
8	193	98	79	33
Total	1410	713	753	223
Mean	176	89	94	28

TABLE 2

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 1 ON HEMOGLOBIN LEVELS

Replications	Treatments			
	Basal	1.0% Mn ($MnSO_4 \cdot H_2O$)	1.0% Mn ($MnCl_2 \cdot 4H_2O$)	1.0% Mn ($MnCO_3$)
	gm./100 ml. blood			
1	11.5	7.3	7.9	7.8
2	12.2	8.7	6.1	6.0
3	12.5	7.3	7.3	7.2
4	12.1	7.5	6.1	6.7
5	12.2	7.5	6.5	5.7
6	12.9	11.8	6.7	6.1
7	12.4	8.0	5.1	4.7
8	11.8	6.3	5.9	8.5
Total	97.5	64.5	51.5	52.7
Mean	12.1	8.1	6.4	6.6

TABLE 3

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 1
ON LIVER COPPER LEVELS

Replications	Treatments			
	Basal	1.0% Mn ($MnSO_4 \cdot H_2O$)	1.0% Mn ($MnCl_2 \cdot 4H_2O$)	1.0% Mn ($MnCO_3$)
	mcg./gm. dry weight			
1	22.2	41.4	22.4	24.5
2	24.6	16.4	14.8	38.9
3	15.3	16.5	15.0	31.1
4	25.2	51.9	19.8	31.5
Total	87.2	126.2	72.0	125.9
Mean	21.8	31.6	18.0	31.5

TABLE 4

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 1
ON LIVER IRON LEVELS

Replications	Treatments			
	Basal	1.0% Mn ($MnSO_4 \cdot H_2O$)	1.0% Mn ($MnCl_2 \cdot 4H_2O$)	1.0% Mn ($MnCO_3$)
	mcg./gm. dry weight			
1	341.9	275.9	122.9	151.7
2	420.2	126.9	114.0	106.9
3	326.0	144.3	111.2	124.9
4	341.0	183.4	125.9	96.3
Total	1429.1	730.5	474.1	479.9
Mean	357.3	182.6	118.5	120.0

TABLE 5

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 1
ON LIVER MANGANESE LEVELS

Replications	Treatments			
	Basal	1.0% Mn ($MnSO_4 \cdot H_2O$)	1.0% Mn ($MnCl_2 \cdot 4H_2O$)	1.0% Mn ($MnCO_3$)
	mcg./gm. dry weight			
1	4.7	71.1	20.9	28.4
2	6.2	49.6	36.3	24.3
3	6.4	57.8	31.1	29.4
4	7.4	117.4	63.8	35.0
Total	24.7	295.8	152.1	117.2
Mean	6.2	74.0	38.0	29.3

TABLE 6

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 1 ON LIVER ZINC LEVELS

Replications	Treatments			
	Basal	1.0% Mn ($MnSO_4 \cdot H_2O$)	1.0% Mn ($MnCl_2 \cdot 4H_2O$)	1.0% Mn ($MnCO_3$)
	mcg./gm. dry weight			
1	50.6	142.0	61.9	29.4
2	49.6	51.0	52.8	62.5
3	29.3	46.8	73.3	75.5
4	52.5	89.3	35.6	64.4
Total	182.1	329.0	223.5	231.7
Mean	45.5	82.3	55.9	57.9

TABLE 7

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2 ON THE GROWTH OF RATS

Replications	Treatments			
	Basal	0.25% Mn	0.5% Mn	1.0% Mn
	4 weeks weight gain (gm.)			
1	203	195	186	88
2	216	188	192	86
3	187	200	182	79
4	209	202	203	120
5	200	207	195	84
6	213	178	157	104
7	198	183	203	117
8	203	168	208	82
Total	1629	1521	1526	760
Mean	204	190	191	95

TABLE 8

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2 ON HEMOGLOBIN LEVELS

Replications	Treatments			
	Basal	0.25% Mn	0.5% Mn	1.0% Mn
	gm./100 ml. blood			
1	13.5	12.5	8.7	8.0
2	12.8	11.5	9.2	7.6
3	12.4	11.8	10.5	6.6
4	13.2	11.9	10.4	7.5
5	12.8	11.8	10.1	7.5
6	13.1	12.4	10.5	8.2
7	12.9	11.8	9.8	8.4
8	13.1	11.8	11.4	7.1
Total	103.8	95.5	80.5	60.8
Mean	13.0	11.9	10.1	7.6

TABLE 9

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2 ON LIVER COPPER LEVELS

Replications	Treatments			
	Basal	0.25% Mn	0.5% Mn	1.0% Mn
	mcg./gm. dry weight			
1	8.6	9.5	4.2	6.9
2	8.4	7.9	7.6	7.9
3	7.0	10.0	7.0	8.6
4	9.5	7.7	8.4	11.0
5	10.6	8.9	10.0	8.0
6	13.0	8.4	8.2	10.3
7	8.4	8.0	7.2	13.5
8	10.5	13.5	9.3	8.0
Total	76.0	73.9	61.9	74.2
Mean	9.5	9.2	7.7	9.3

TABLE 10

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2 ON LIVER IRON LEVELS

Replications	Treatments			
	Basal	0.25% Mn	0.5% Mn	1.0% Mn
	mcg./gm. dry weight			
1	246.2	129.9	136.3	54.4
2	213.9	128.4	55.6	43.8
3	156.1	121.2	44.9	62.4
4	263.4	126.3	86.3	75.8
5	181.3	137.7	136.9	54.1
6	309.7	136.9	70.5	64.3
7	221.7	133.8	91.6	68.4
8	189.9	123.8	82.9	38.0
Total	1782.2	1038.0	705.0	461.2
Mean	222.8	129.8	88.1	57.7

TABLE 11

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2
ON LIVER MANGANESE LEVELS

Replications	Treatments			
	Basal	0.25% Mn	0.5% Mn	1.0% Mn
	mcg./gm. dry weight			
1	2.7	5.5	12.2	8.4
2	3.9	5.8	9.3	8.4
3	4.4	6.6	11.7	27.8
4	3.5	8.6	8.3	24.3
5	3.6	9.4	10.8	18.0
6	4.2	6.6	14.8	16.3
7	4.5	8.2	10.7	55.2
8	3.8	10.7	14.0	16.5
Total	30.6	61.4	91.7	174.7
Mean	3.8	7.7	11.5	21.8

TABLE 12

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2 ON LIVER ZINC LEVELS

Replications	Treatments			
	Basal	0.25% Mn	0.5% Mn	1.0% Mn
	mcg./gm. dry weight			
1	57.5	60.8	56.5	48.0
2	50.5	58.5	53.3	38.4
3	61.5	64.7	62.1	48.4
4	71.9	59.0	51.3	54.8
5	55.4	72.2	53.3	54.0
6	59.3	66.5	55.5	48.4
7	127.1	61.0	58.0	44.9
8	53.4	64.2	58.6	47.0
Total	536.6	507.0	448.4	383.7
Mean	67.1	63.4	56.1	48.0

TABLE 13

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2 ON BONE CALCIUM LEVELS

Replications	Treatments			
	Basal	0.25% Mn	0.5% Mn	1.0% Mn
	mg./gm. dry weight			
1	1321.6	399.9	2442.9	4562.8
2	580.4	1423.0	789.9	2623.4
3	524.9	742.9	557.8	2469.5
4	501.7	541.6	924.7	815.8
5	414.6	439.1	488.6	2450.9
6	469.1	571.4	1302.2	2238.3
7	1991.4	1039.5	649.2	4328.1
8	2571.0	667.0	472.9	852.7
Total	8374.6	5824.4	7628.3	20341.6
Mean	1046.8	728.0	953.5	2542.7

TABLE 14

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2
ON BONE MAGNESIUM LEVELS

Replications	Treatments			
	Basal	0.25% Mn	0.5% Mn	1.0% Mn
	mg./gm. dry weight			
1	5.1	3.7	3.5	2.1
2	4.2	4.4	3.9	1.9
3	4.6	4.0	3.5	1.8
4	3.5	4.2	3.6	1.8
5	2.2	2.2	3.0	2.3
6	3.5	2.7	2.2	2.2
7	2.6	3.0	3.5	1.8
8	2.4	2.4	2.5	1.5
Total	28.1	26.5	25.6	15.3
Mean	3.5	3.3	3.2	1.9

TABLE 15

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2
ON BONE MANGANESE LEVELS

Replications	Treatments			
	Basal	0.25% Mn	0.5% Mn	1.0% Mn
	mcg./gm. dry weight			
1	15.8	15.7	15.3	31.6
2	10.0	12.5	16.7	28.1
3	12.3	13.5	12.3	27.6
4	12.0	13.1	12.9	27.0
5	11.5	13.0	13.4	28.1
6	15.3	13.8	18.1	27.3
7	11.5	13.3	13.7	25.0
8	12.1	11.8	14.0	26.7
Total	100.3	106.7	116.4	221.4
Mean	12.5	13.3	14.6	27.7

TABLE 16

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2
ON BONE PHOSPHORUS LEVELS

Replications	Treatments			
	Basal	0.25% Mn	0.5% Mn	1.0% Mn
	mg./gm. dry weight			
1	139.8	91.7	121.1	165.9
2	79.9	94.1	92.1	106.7
3	63.9	52.2	81.8	195.9
4	67.2	43.2	87.0	88.4
5	73.3	55.7	86.5	134.8
6	57.0	75.2	106.1	191.0
7	114.4	116.6	61.2	203.7
8	110.6	54.9	94.3	157.2
Total	706.2	583.6	730.2	1243.6
Mean	88.3	72.9	91.3	155.5

TABLE 17

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON GROWTH OF RATS

Treatments

1. Basal
2. 1.0% Manganese
3. 1.0% Mn + 10% Distiller's dried solubles
4. 1.0% Mn + 0.8% Ca + 0.8% P
5. 1.0% Mn + 10% Soybean meal
6. 1.0% Mn + 10% Extra casein
7. 1.0% Mn + 10% Alpha protein
8. 1.0% Mn + 10% Lactalbumin
9. 1.0% Mn + 10% Blood albumin
10. 1.0% Mn + 0.02% Cu + 0.04% Fe

Reps	Treatments 1	2	3	4	5	6	7	8	9	10
	4 weeks weight gain (gm.)									
1	163	112	158	66	112	106	43	32	96	7
2	193	145	147	154	140	36	62	90	63	96
3	197	129	155	100	146	57	42	42	103	34
4	137	138	147	141	144	107	(42)[a]	11	76	35
5	199	130	156	157	143	131	80	75	72	1
6	195	112	157	41	133	117	13	117	93	59
Total	1084	766	920	659	818	554	282	367	503	232
Mean	181	128	153	110	136	92	47	61	84	39

[a] ( ) indicates calculated missing plot value.

TABLE 18

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON HEMOGLOBIN LEVELS

Treatments

1. Basal
2. 1.0% Manganese
3. 1.0% Mn + 10% Distiller's dried solubles
4. 1.0% Mn + 0.8% Ca + 0.8% P
5. 1.0% Mn + 10% Soybean meal
6. 1.0% Mn + 10% Extra casein
7. 1.0% Mn + 10% Alpha protein
8. 1.0% Mn + 10% Lactalbumin
9. 1.0% Mn + 10% Blood albumin
10. 1.0% Mn + 0.02% Cu + 0.04% Fe

Reps	Treatments 1	2	3	4	5	6	7	8	9	10
					gm./100 ml. blood					
1	12.7	8.7	8.1	9.1	7.5	8.3	7.8	9.2	8.7	15.5
2	13.0	8.7	8.4	8.1	8.2	7.8	7.8	7.9	8.7	12.8
3	12.8	8.2	8.8	8.5	7.8	8.3	7.7	7.8	9.5	13.8
4	11.5	11.1	8.3	8.3	8.9	8.3	13.4	9.6	7.9	11.5
5	12.3	7.6	9.7	8.7	6.5	7.9	7.5	7.9	7.7	12.4
6	12.8	8.2	8.5	9.8	7.5	8.1	9.1	8.1	10.1	11.2
Total	75.0	52.5	52.0	52.4	46.5	48.8	53.3	50.6	52.6	77.1
Mean	12.5	8.8	8.7	8.7	7.7	8.1	8.9	8.4	8.8	12.9

TABLE 19

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON LIVER COPPER LEVELS

Treatments

1. Basal
2. 1.0% Manganese
3. 1.0% Mn + 10% Distiller's dried solubles
4. 1.0% Mn + 0.8% Ca + 0.8% P
5. 1.0% Mn + 10% Soybean meal
6. 1.0% Mn + 10% Extra casein
7. 1.0% Mn + 10% Alpha protein
8. 1.0% Mn + 10% Lactalbumin
9. 1.0% Mn + 10% Blood albumin
10. 1.0% Mn + 0.02% Cu + 0.04% Fe

Reps	Treatments 1	2	3	4	5	6	7	8	9	10
					mcg./gm. dry weight					
1	20.6	15.1	25.3	20.1	13.3	25.2	56.9	17.0	19.5	1048.5
2	15.8	13.1	12.5	14.7	9.7	17.4	30.3	12.3	18.7	946.7
3	19.7	29.6	22.1	21.2	15.4	19.7	33.9	18.0	19.5	832.1
4	13.9	19.9	23.1	11.2	15.5	19.9	40.4	63.8	15.8	1193.5
Total	70.0	77.7	82.9	67.2	53.9	82.2	151.5	111.1	73.5	4020.8
Mean	17.5	19.4	20.7	16.8	13.5	20.6	37.9	27.8	18.4	1005.2

TABLE 20

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON LIVER IRON LEVELS

Treatments

1. Basal
2. 1.0% Manganese
3. 1.0% Mn + 10% Distiller's dried solubles
4. 1.0% Mn + 0.8% Ca + 0.8% P
5. 1.0% Mn + 10% Soybean meal
6. 1.0% Mn + 10% Extra casein
7. 1.0% Mn + 10% Alpha protein
8. 1.0% Mn + 10% Lactalbumin
9. 1.0% Mn + 10% Blood albumin
10. 1.0% Mn + 0.02% Cu + 0.04% Fe

Reps	Treatments 1	2	3	4	5	6	7	8	9	10
	mcg./gm. dry weight									
1	337.2	85.4	82.0	92.9	84.3	87.2	98.9	103.1	107.6	320.5
2	242.0	108.2	87.2	72.9	90.7	93.2	104.8	91.4	100.5	339.6
3	334.2	95.4	85.2	92.3	80.9	93.5	85.9	105.0	92.4	274.4
4	291.4	98.2	83.8	93.0	87.8	94.9	109.9	135.1	85.8	242.0
Total	1204.8	387.2	338.1	351.1	343.7	368.8	399.5	434.7	386.3	1176.5
Mean	301.2	96.8	84.5	87.8	85.9	92.2	99.9	108.7	96.6	294.1

TABLE 21

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON LIVER MANGANESE LEVELS

Treatments

1. Basal
2. 1.0% Manganese
3. 1.0% Mn + 10% Distiller's dried solubles
4. 1.0% Mn + 0.8% Ca + 0.8% P
5. 1.0% Mn + 10% Soybean meal
6. 1.0% Mn + 10% Extra casein
7. 1.0% Mn + 10% Alpha protein
8. 1.0% Mn + 10% Lactalbumin
9. 1.0% Mn + 10% Blood albumin
10. 1.0% Mn + 0.02% Cu + 0.04% Fe

	Treatments									
Reps	1	2	3	4	5	6	7	8	9	10
	mcg./gm. dry weight									
1	4.7	31.7	14.8	10.5	24.8	24.2	57.0	18.1	39.0	49.6
2	4.6	41.2	20.0	9.7	19.4	27.0	41.0	24.3	32.3	19.8
3	5.2	22.2	21.6	9.1	20.9	9.7	35.9	39.3	41.6	40.8
4	4.3	23.9	16.7	9.4	21.2	16.4	31.8	43.5	31.1	30.2
Total	18.7	119.0	73.2	38.7	86.3	77.3	165.7	125.1	143.9	140.4
Mean	4.7	29.7	18.3	9.7	21.6	19.3	41.4	31.3	36.0	35.1

TABLE 22

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON LIVER ZINC LEVELS

Treatments

1. Basal
2. 1.0% Manganese
3. 1.0% Mn + 10% Distiller's dried solubles
4. 1.0% Mn + 0.8% Ca + 0.8% P
5. 1.0% Mn + 10% Soybean meal
6. 1.0% Mn + 10% Extra casein
7. 1.0% Mn + 10% Alpha protein
8. 1.0% Mn + 10% Lactalbumin
9. 1.0% Mn + 10% Blood albumin
10. 1.0% Mn + 0.02% Cu + 0.04% Fe

Reps	Treatments 1	2	3	4	5	6	7	8	9	10
	mcg./gm. dry weight									
1	89.3	87.9	83.6	86.6	93.1	78.2	100.6	103.2	77.3	343.6
2	70.6	72.9	75.8	66.8	80.9	93.2	81.8	87.3	69.4	79.9
3	61.4	75.7	68.8	82.9	71.0	77.3	94.0	75.1	83.0	76.3
4	72.7	91.0	58.1	94.0	73.5	164.0	318.0	89.1	310.7	102.7
Total	294.0	327.5	286.2	330.3	318.4	412.8	594.4	354.7	540.3	602.5
Mean	73.5	81.9	71.6	82.6	79.6	103.2	148.6	88.7	135.1	150.6

TABLE 23

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON BONE CALCIUM LEVELS

Treatments

1. Basal
2. 1.0% Manganese
3. 1.0% Mn + 10% Distiller's dried solubles
4. 1.0% Mn + 0.8% Ca + 0.8% P
5. 1.0% Mn + 10% Soybean meal
6. 1.0% Mn + 10% Extra casein
7. 1.0% Mn + 10% Alpha protein
8. 1.0% Mn + 10% Lactalbumin
9. 1.0% Mn + 10% Blood albumin
10. 1.0% Mn + 0.02% Cu + 0.04% Fe

Reps	Treatments 1	2	3	4	5	6	7	8	9	10
	mg./gm. dry weight									
1	195.7	204.6	202.7	218.6	176.3	176.6	83.9	90.4	143.2	160.4
2	229.6	235.6	195.8	247.5	186.3	216.2	222.2	186.0	196.8	193.3
3	217.9	217.9	229.5	206.7	207.3	199.8	132.9	203.9	186.6	175.7
Total	643.2	658.1	628.0	672.8	569.9	592.6	439.0	480.3	526.6	529.4
Mean	214.4	219.4	209.3	224.3	189.9	197.5	146.3	160.1	175.5	176.4

TABLE 24

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON BONE MAGNESIUM LEVELS

Treatments

1. Basal
2. 1.0% Manganese
3. 1.0% Mn + 10% Distiller's dried solubles
4. 1.0% Mn + 0.8% Ca + 0.8% P
5. 1.0% Mn + 10% Soybean meal
6. 1.0% Mn + 10% Extra casein
7. 1.0% Mn + 10% Alpha protein
8. 1.0% Mn + 10% Lactalbumin
9. 1.0% Mn + 10% Blood albumin
10. 1.0% Mn + 0.02% Cu + 0.04% Fe

Reps	Treatments 1	2	3	4	5	6	7	8	9	10
	mg./gm. dry weight									
1	1315.0	1674.5	1881.0	1463.0	1343.8	2233.9	1241.6	1146.7	1374.4	1526.0
2	1318.1	1574.3	1296.0	1469.4	1403.9	1705.4	1966.8	1403.3	2062.1	1597.9
3	1401.7	1550.9	1499.1	1511.6	1932.5	1813.2	1801.0	1785.0	1943.5	2042.5
Total	4034.8	4799.6	4676.0	4444.1	4680.2	5752.5	5009.5	4335.0	5380.0	5166.4
Mean	1344.9	1599.9	1558.7	1481.4	1560.1	1917.5	1669.8	1445.0	1793.3	1722.1

TABLE 25

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON BONE MANGANESE LEVELS

Treatments

1. Basal
2. 1.0% Manganese
3. 1.0% Mn + 10% Distiller's dried solubles
4. 1.0% Mn + 0.8% Ca + 0.8% P
5. 1.0% Mn + 10% Soybean meal
6. 1.0% Mn + 10% Extra casein
7. 1.0% Mn + 10% Alpha protein
8. 1.0% Mn + 10% Lactalbumin
9. 1.0% Mn + 10% Blood albumin
10. 1.0% Mn + 0.02% Cu + 0.04% Fe

Reps	Treatments 1	2	3	4	5	6	7	8	9	10
	mcg./gm. dry weight									
1	14.9	19.1	20.6	15.1	23.1	25.7	50.6	45.4	40.8	38.2
2	12.1	42.5	21.4	21.4	27.1	23.6	56.7	40.9	44.1	(37.6)[a]
3	13.5	50.7	20.3	23.8	22.8	28.6	49.8	41.4	36.5	33.0
Total	40.5	112.3	62.3	60.4	73.0	77.9	157.1	127.8	121.4	108.8
Mean	13.5	37.4	20.8	20.1	24.4	26.0	52.4	42.6	40.5	36.3

[a] ( ) indicates calculated missing plot value.

TABLE 26

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON BONE PHOSPHORUS LEVELS

Treatments

1. Basal
2. 1.0% Manganese
3. 1.0% Mn + 10% Distiller's dried solubles
4. 1.0% Mn + 0.8% Ca + 0.8% P
5. 1.0% Mn + 10% Soybean meal
6. 1.0% Mn + 10% Extra casein
7. 1.0% Mn + 10% Alpha protein
8. 1.0% Mn + 10% Lactalbumin
9. 1.0% Mn + 10% Blood albumin
10. 1.0% Mn + 0.02% Cu + 0.04% Fe

Reps	Treatments 1	2	3	4	5	6	7	8	9	10
	mg./gm. dry weight									
1	100.5	87.0	69.0	31.9	82.2	72.2	149.3	23.7	21.6	54.8
2	26.9	47.5	44.3	32.1	51.3	31.3	26.1	61.8	59.6	24.4
3	69.1	55.2	31.4	30.0	39.9	36.1	65.5	56.1	47.5	89.3
Total	196.6	189.7	144.7	94.0	173.4	139.6	240.9	141.6	128.7	168.5
Mean	65.5	63.2	48.2	31.3	57.8	46.5	80.3	47.2	42.9	56.2

APPENDIX B

ANALYSIS OF VARIANCE DATA

TABLE 1

ANALYSES OF VARIANCE OF DATA COLLECTED IN EXPERIMENT 1

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Weight Gain	
Total	31	111,996	
Replications	7	7,218	
Treatments	3	89,032	29,677**
Error	21	15,747	750
		Hemoglobin Level	
Total	31	209.35	
Replications	7	8.34	
Treatments	3	171.58	57.19**
Error	21	29.43	1.40
		Liver Copper	
Total	15	2,248.65	
Replications	3	329.09	
Treatments	3	1,077.41	359.14
Error	9	842.15	93.57

TABLE 1--Continued

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Liver Iron	
Total	15	172,478.19	
Replications	3	4,825.83	
Treatments	3	151,846.95	50,615.65**
Error	9	15,805.41	1,756.16
		Liver Manganese	
Total	15	13,321.77	
Replications	3	1,947.29	
Treatments	3	9,507.67	3,169.22**
Error	9	1,866.81	207.42
		Liver Zinc	
Total	15	11,146.09	
Replications	3	713.39	
Treatments	3	2,904.51	968.17*
Error	9	7,528.19	836.47

*Significant ($p \leq 0.05$).

**Highly significant ($p \leq 0.01$).

TABLE 2

ANALYSES OF VARIANCE OF DATA COLLECTED IN EXPERIMENT 2

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square
		Weight Gain	
Total	31	66,282	
Replications	7	1,407	
Treatments	3	60,729	20,243**
Error	21	4,145	197
		Hemoglobin Level	
Total	31	142.56	
Replications	7	1.92	
Treatments	3	133.73	44.58**
Error	21	6.91	.33
		Liver Copper	
Total	31	119.64	
Replications	7	30.52	
Treatments	3	15.68	5.22
Error	21	73.44	3.49

TABLE 2--Continued

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square
		Liver Iron	
Total	31	150,247.5	
Replications	7	8,770.4	
Treatments	3	123,820.6	41,273.53**
Error	21	17,656.5	840.78
		Liver Manganese	
Total	31	3,093.95	
Replications	7	436.85	
Treatments	3	1,441.06	480.35*
Error	21	1,216.04	57.91
		Liver Zinc	
Total	31	6,539.96	
Replications	7	1,156.00	
Treatments	3	1,713.36	571.12
Error	21	3,670.60	174.79

TABLE 2--Continued

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square
		Bone Calcium	
Total	31	39,907,971.61	
Replications	7	7,423,421.41	
Treatments	3	16,400,248.85	5,466,749.61**
Error	21	14,084,301.35	670,681.01
		Bone Phosphorus	
Total	31	57,617.28	
Replications	7	10,038.43	
Treatments	3	32,409.29	10,803.09*
Error	21	15,169.56	722.36
		Bone Magnesium	
Total	31	28.11	
Replications	7	8.53	
Treatments	3	12.60	4.20**
Error	21	6.98	.33
		Bone Manganese	
Total	31	1,315.60	
Replications	7	50.59	
Treatments	3	1,226.40	408.80**
Error	21	38.61	1.84

*Significant ($p \leq 0.05$).

**Highly significant ($p \leq 0.01$).

TABLE 3

ANALYSES OF VARIANCE OF DATA COLLECTED IN EXPERIMENT 3

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Weight Gain	
Total	58	160,683	
Replications	5	4,381	
Treatments	9	119,026	13,225**
Error	45	37,275	828
		Hemoglobin Level	
Total	59	235.55	
Replications	5	6.68	
Treatments	9	173.18	19.24**
Error	45	55.69	1.24
		Liver Copper	
Total	39	3,557,253.30	
Replications	3	9,120.70	
Treatments	9	3,483,736.79	387,081.87**
Error	27	64,395.81	2,385.03

TABLE 3--Continued

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Liver Iron	
Total	39	254,256.71	
Replications	3	2,140.59	
Treatments	9	240,106.09	26,678.45**
Error	27	12,010.03	444.82
		Liver Manganese	
Total	39	6,997.79	
Replications	3	114.40	
Treatments	9	5,157.90	1,684.21**
Error	27	1,725.49	63.91
		Liver Zinc	
Total	39	172,187.21	
Replications	3	26,338.46	
Treatments	9	35,321.88	3,924.65
Error	27	110,526.87	4,093.59

TABLE 3--Continued

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Bone Calcium	
Total	29	42,041.34	
Replications	2	11,069.69	
Treatments	9	18,713.74	2,079.30**
Error	18	12,257.91	680.99
		Bone Phosphorus	
Total	29	23,010.89	
Replications	2	2,936.23	
Treatments	9	15,103.33	1,678.15
Error	18	4,971.66	276.20
		Bone Magnesium	
Total	29	2,269,909.94	
Replications	2	229,704.20	
Treatments	9	790,726.65	87,858.52
Error	18	1,249,489.09	69,416.06
		Bone Manganese	
Total	28	4,730.24	
Replications	2	64.13	
Treatments	9	4,037.79	448.64**
Error	17	628.32	36.96

*Significant ($p \leq 0.05$).

**Highly significant ($p \leq 0.01$).